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# Pharmacological Interventions of Selenium in Duchene Muscular Dystrophy: The Role of Reactive Oxygen Species in Mediating Lipid Peroxide Formation

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Additional information is available at the end of the chapter

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## 1. Introduction

The muscular dystrophies are a group of muscle diseases which have three features in common: they are hereditary; they are progressive; and each causes a characteristic, selective pattern of weakness. Duchenne muscular dystrophy (DMD) is the most common inherited muscular disease, affecting one in 3,500 male births. DMD is characterized by rapid, progressive muscle wasting that typically kills patients in their twenties. Complete muscle dystrophin deficiency is a common mechanism for DMD.

Duchenne muscular dystrophy (DMD) is a severe X-linked recessive, progressive muscle-wasting disease affecting one in 3500 boys (Emery AEH, 1993). Patients are usually confined to a wheelchair before the age of 12 and die in their late teens or early twenties usually of respiratory failure. A milder form of the disease, Becker muscular dystrophy (BMD), has a later onset and a much longer survival.

The most widely studied dystrophies are those due to mutations in the dystrophin gene. In humans, dystrophin deficiency leads to the severe disease, Duchenne muscular dystrophy, whereas reductions or truncations of dystrophin lead to a milder disease, Becker muscular dystrophy. Numerous animal models of dystrophin deficiency are actively studied, but none more so than the *mdx* mouse. Despite over a decade of study of dystrophin and its associated proteins, some of which themselves cause muscular dystrophies when deficient or defective, the mechanisms by which the primary biochemical defects lead to muscle cell death remain to be determined.

Both disorders are caused by mutations in the DMS gene that encodes a 427-kDa cytoskeletal protein called dystrophin. The vast majority of DMD mutations result in the complete absence of dystrophin, whereas the presence of low levels of a truncated protein is seen in BMD patients. The defected gene causes a shortage or absence of the structural protein dystrophin, which is near the sites of  $\text{Ca}^{2+}$  release from sarcoplasmic reticulum and uptake of intracellular  $\text{Ca}^{2+}$ . The genetic alteration produces an abnormality in the membrane of muscular fibers that consists of a disturbance in the calcium transport ( $\text{Ca}^{++}$ ), inside the muscular fibers, which is the base mechanism of cellular degeneration, necrosis, and apoptosis (Simonian and Coyle 1996). A nucleotide degeneration and decreased muscle AP and ADP content has been reported. Of the total body selenium reserves consists of muscle selenium supply. Selenium is gradually wasted out by the kidneys during the proceeding of the dystrophy of the legs (Westermarck et al 1982). Open follow-up trials with antioxidants are indicating positive clinical response (Gebre-Medhin et al 1985; Timberg 1989; Westermarck et al 1997).

## 2. Description and clinical features of Duchenne

Typically, DMD patients are clinically normal at birth, although serum levels of muscle isoform of creatine kinase are elevated. The onset of pseudohypertrophy of the calf muscles, proximal limb muscle weakness suggests DMD. Weakness of the arms occurs later along with progressive kyphoscoliosis (Dubowitz. Major Probl Clin Pediatr 1978).

Most patients die in their early twenties as a result of respiratory complications due to intercostal muscle weakness and respiratory infection. Death can also be the result of cardiac dysfunction with cardiomyopathy. BMD and DMD patients also present with mild cognitive impairment, indicating that brain function is also abnormal in these disorders (Blak and Martin-Rendon, 2002).

Fetal DMD is histologically normal except for occasional eosinophilic hypercontracted fibers (Bertorini et al. 1984). Necrotic or degenerating muscle fibers are seen in all postnatal DMD muscle biopsies even before muscle weakness is clinically seen. The necrotic fibers are phagocytized, and muscle biopsies from DMD patients reveal the presence of inflammatory cells at perimysial and endomysial sites (Arahata and Engel 1984). The regenerative capacity of the muscle is lost and muscle fibers are gradually replaced by adipose and fibrous connective tissue, giving rise to the clinical appearance of pseudohypertrophy followed by atrophy (Emery 1993), resulting in muscle wasting and ultimately muscle weakness (Blake et al 2002). Most patients die in their early twenties as a result of respiratory complications due to intercostal muscle weakness and respiratory infection. Death can also be the result of cardiac dysfunction with cardiomyopathy. BMD and DMD patients also present with mild cognitive impairment, indicating that brain function is also abnormal in these disorders (Blake and Kroger 2000).

DMD muscle shows signs of oxidative damage (Murphy and Kehrer 1989). Muscle diseases in which oxidative damage may play a primary role show features in common with DMD (Mendell Et al 1971). Moreover, muscle cells from mdx mice have an increased susceptibility

to oxidative stress (Rando et al 1998). The lack of neuronal-type nitric oxide synthase (nNOS) seen in DMD causes dysregulation of vascular tone, and ischemia (Crosbie 2001, Blake et al 2002). This phenomenon may increase the generation of free radicals. Open follow-up trials with antioxidants are indicating positive clinical response (Gebre-Medhin et al 1985; Timber 1989; Westermarck et al 1997).

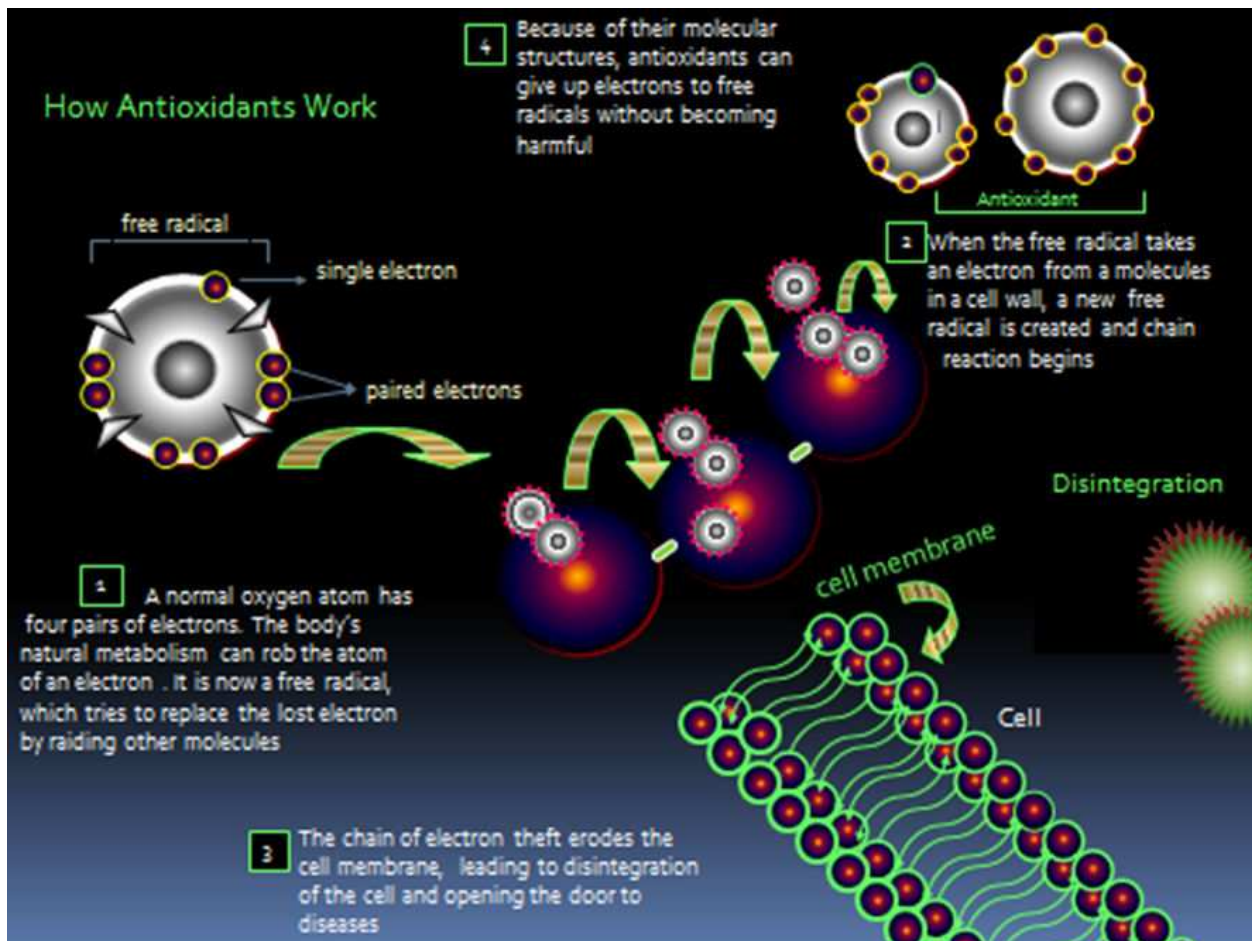
A low blood selenium level has previously been observed in healthy inhabitants of Finland (WESTERMARCK, 1977). In this study even lower blood selenium values were observed in patients with acrodermatitis enteropathica, dystrophia musculorum progressiva (Duchenne), infantile and juvenile type of neuronal ceroid lipofuscinosis (NCL), severe mental retardation caused by various factors, and myocardial infarction. The selenium content of the brain, heart, kidney and liver in patients of different ages was also determined. The highest selenium level was found in the kidney. The mean liver selenium concentrations in stillborn, premature and full-term neonates were 1.11 +/- 0.23 (8), 1.21 +/- 0.17 (12) and 0.93 +/- 0.16 microgram/g dry weight (12) respectively (the number of subjects in parentheses). The selenium values are considerably higher than those in infants of from one to nine months of age and adults, whose liver selenium values were 0.58 +/- 0.21 (8) and 0.67 +/- 0.08 microgram/g dry weight (8) respectively. The vitamin E levels of serum in patients with NCL, as well as in subjects with severe mental retardation (controls), were low compared with values in healthy normal subjects. Sodium selenite supplementation in patients with NCL produced at least a transitory improvement without causing any toxic effects during one year of administration. In Duchenne muscular dystrophy we found that <sup>75</sup>Se-selenite was not absorbed in the lower extremities in the equal level compared to healthy ones.

### 3. Oxidative stress

Oxidative stress is often defined as an imbalance between the generation of reactive oxygen species and the removal of such species by enzymatic and nonenzymatic cellular defense systems (Figure 1). This imbalance could arise from overproduction of reactive species, as occurs under certain pathologic conditions and in association with inflammation, or from an impairment of the defense mechanisms, as occurs in certain genetic loss-of-function disorders and deficiency states. Implicit in this definition is the notion that such an imbalance is sufficient to lead to the oxidation of various cellular constituents and to cause cellular dysfunction and injury. As such, oxidative stress may also be viewed as a condition in which the production of oxidative products exceeds their removal by cellular repair mechanisms. Such conditions may lead to acute cellular dysfunction or cell death, and chronic tissue degeneration, if such changes accumulate.

During normal cellular metabolism, the primary generation of reactive oxygen species comes from the leakage of superoxide anions from the electron transport chain. A series of linked enzymatic reactions are responsible for the detoxification of superoxide. Superoxide is converted to hydrogen peroxide by the action of superoxide dismutase (SOD). Most animal cells contain two forms of SOD, a cytoplasmic Cu,Zn-SOD and a mitochondrial Mn-SOD. In

addition, there is an extracellular form of the enzyme. Hydrogen peroxide is subsequently metabolized to oxygen and water by the selenium-containing enzyme glutathione peroxidase, which uses glutathione (GSH) as a cofactor in the reaction. Glutathione peroxidase converts most of the hydrogen peroxide in the cytoplasm. At sites of relatively high concentrations of hydrogen peroxide, such as peroxisomes, catalase is an important antioxidant enzyme that also converts hydrogen peroxide to water. Hydrogen peroxide can react with metal ions in the cell to produce the highly reactive hydroxyl radical, and superoxide can react with nitric oxide (NO•) to produce peroxynitrite. Hydroxyl radical and peroxynitrite are among the most reactive species present in biological systems and are capable of oxidizing nucleic acids, proteins, lipids, and carbohydrate moieties in the cell.



**Figure 1.** The role of free radical in inflammation

#### 4. Oxidative stress cause dystrophic changes

One of the first observations that led to the oxidative stress hypothesis was the finding that vitamin E deficiency in animals leads to muscle degeneration with pathologic characteristics

very similar to those of the muscular dystrophies. The similarities are most striking in avian species in which vitamin E deficiency myopathies closely mimic the hereditary dystrophies both anatomically and biochemically. In humans, vitamin E deficiency is associated with myopathic changes, and in these disorders, there is selective involvement of type IIB fibers as in the inherited muscular dystrophies. Vitamin E refers to a group of compounds of which [alpha]-tocopherol is the most potent and most prevalent in animal tissues as the major lipid-soluble antioxidant in the cell. Deficiencies of vitamin E are associated with increases in lipid peroxidation and decreases in polyunsaturated fatty acids in muscle and compensatory increases in muscle antioxidant enzymes and GSH levels. Although it is clear that inherited muscular dystrophies are not due to primary deficiencies in vitamin E, as was once proposed, the cumulative data strongly support the proposition that the mechanism of muscle injury is the same in both conditions.

## 5. Can antioxidant treatment ameliorate muscular dystrophy

In addition to evidence of oxidative damage preceding pathologic changes, amelioration of the pathology of a muscular dystrophy by antioxidant treatment would be strong support for the hypothesis that oxidative stress is a primary pathogenetic process. Various antioxidant treatments have been tried in humans and animals with muscular dystrophy. However, the benefit from any individual antioxidant treatment would depend on the actual nature of the oxidative stress that is occurring in the muscle tissue. For example, supplementation of vitamin E-deficient animals with the most prevalent cellular soluble antioxidant, ascorbic acid (vitamin C), does not significantly improve the myopathy. Different susceptibilities to oxidative stress are not identical. Even if oxidative stress is indeed the primary pathophysiologic process leading to muscle cell death in the dystrophies, effective treatment will need to be targeted to the specific deficit in antioxidant defense in the dystrophic muscle and thus will depend on a detailed understanding of the nature of that susceptibility.

Antioxidant treatments in animals with hereditary muscular dystrophies have provided modest benefits. Penicillamine, a sulfhydryl compound with antioxidant properties, and vitamin E slowed the degenerative process in avian dystrophy. Research showed that iron deprivation resulted in a significant reduction of necrosis in the mdx mouse, presumably by a decrease in the production of hydroxyl radical. Dietary supplementation rich in antioxidants significantly reduced an index of muscle weakness in mdx mice.

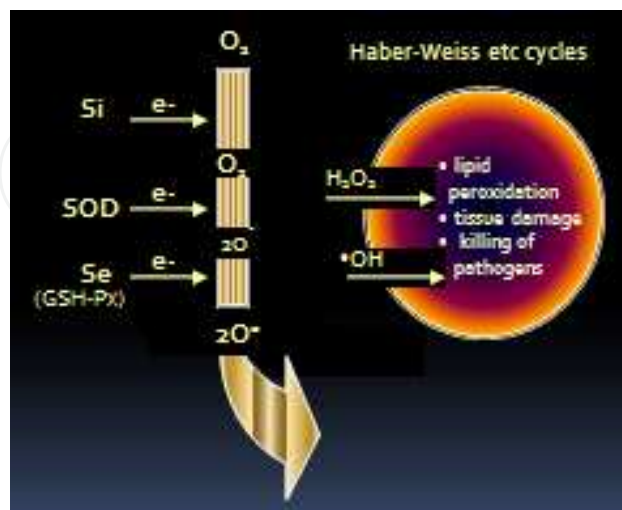
Clinical trials of antioxidant therapy in humans with Duchenne muscular dystrophy have included treatments with tocopherols, ascorbate, penicillamine, and SOD. No clear benefit has been found from any of these treatments. However, these trials have been very limited in duration and size. Furthermore, in no human study has antioxidant treatment begun early in the course of the disease. In fact, all of these studies involved boys with advanced disease (average age,  $\geq 10$  yr). Based on the notion that oxidative injury is critical to the pathogenesis of muscle cell death and that antioxidant treatment might be effective to prevent such death, trials in humans would need to be initiated early in the course of the disease, and efficacy

would need to be assessed primarily as the slowing, not a reversal, of muscle loss. The difficulties and pitfalls of clinical trials for new treatments of muscular dystrophies are well known. Thus, based on both statistical power and theoretical benefit, none of these human trials would even be predicted to demonstrate any benefit, and the negative results do not in any way refute the oxidative stress hypothesis.

## 6. Selenium in the brain and brain diseases

Oxidative stress and generation of reactive oxygen species (ROS) are strongly implicated in a number of neuronal and neuromuscular disorders, including stroke and cerebrovascular disease, Alzheimer's disease, Parkinson's disease, familial amyotrophic lateral sclerosis, and Duchenne muscular dystrophy (Dexter et al. 1989a,b; Smith et al. 1991; Ragusa et al. 1997; Cornett et al. 1998b; Facchinetti et al. 1998; Sagara et al. 1998; Tan et al. 1998). Selenium is known to provide protection from ROS-induced cell damage, and the proposed mechanisms mainly invoke the functions of glutathione peroxidases (GPxs) and selenoprotein P (SelP).

Considerable evidence exists linking heavy metals to neurodegenerative diseases (Thompson et al. 1988; Deibel et al. 1996; Cuajungco and Lees 1997; Schionning et al. 1997; Cornett et al. 1998b; Ely 2001). Heavy metals trigger the conversion of hydrogen peroxide to hydroxyl radical through Fenton reaction. Selenium has long been known to function as an antidote to toxicity of heavy metals. Co-administration of selenium was reported to play a role in reducing the toxic effects of mercury as early as the 1970s (Koelman et al. 1973; Kosta et al. 1975). Among the selenoproteins, SelP has been reported in several studies to possess metal-binding function (see below). The GPxs might also detoxify heavy metals through their well-known function of eliminating peroxides (Figure 2).



**Figure 2.** Complementary actions of selenium (GSH-Px) in free radical formation and lipid peroxidation (from Parantainen et al., 1987)

## 7. Treatment of brain diseases with selenium

Two children with severe neurodevelopmental retardation and elevated liver function tests developed intractable seizures during the first years of life. They were found systemically selenium deficient. Oral substitution with selenium supplements in both children (3–5 µg/kg body weight) resulted in reduction of seizures, improvement of the electroencephalogram (EEG) recordings, and return of normal liver function after 2 weeks (Ramaekers et al. 1994). It is unknown if selenium deficiency is a direct factor for the neurodevelopmental retardation or it affected the brain via abnormal liver function.

Methamphetamine (MA) exposure of animals results in enhanced formation of superoxide radical ( $O_2^-$ ) and nitric oxide (NO), which interact to produce peroxynitrite ( $OONO^-$ ). Peroxynitrite is a potent oxidant, leading to dopaminergic damage (Imam and Ali 2000). Thus, multiple dose administration of MA to mice results in long-lasting toxic effects in the nigrostriatal dopaminergic system, which is a relevant model of PD. In selenium-replete mice, this dopaminergic toxicity was significantly attenuated, compared with selenium-deficient mice (Kim et al. 1999; Kim et al. 2000). Pre-treatment of animals with selenium and melatonin can completely protect against the depletion of striatal dopamine induced by MA exposure (Imam et al. 2001). The reason for the protective effects of selenium against MA was reported to be the efficient scavenging of peroxynitrite by selenoproteins (Sies and Arteel 2000).

6-hydroxydopamine (6-OHDA) is a neurotoxin specific for catecholamine neurons in both the central and peripheral nervous system. PD induced by this compound in rats was prevented by selenium in a dose-dependent manner, through up-regulating the antioxidant status and lowering the dopamine loss. This study revealed that selenium may be helpful in slowing down the progression of neurodegeneration in Parkinsonism (Zafar et al. 2003).

## 8. Hypothesis and intervention

It is possible to improve the life quality of Duchenne patients by biological active substances (antioxidants), and psychotherapeutic intervention. Therapy of DMD has been an elusive goal. Studies with isolated myocytes have shown that lipid peroxidation with an enhanced free radical production can be activated by increasing Ca concentration. No wonder that several kinds of antioxidants have been proposed as a treatment since increased levels of thiobarbituric acid (TBA) reactive material has been found in the muscles and blood of patients with DMD.

Vitamin E has been observed to decrease the amount of TBA reactive material in dystrophic muscle. Previously we have reported that the biological half-life of selenium-75 ( $^{75}Se$ ) in DMD patients is significantly shorter than in healthy controls (Westermarck et al 1982). On the basis of these facts we started to treat some DMD patients with selenium and other antioxidants. Selenium is activating a well-known antioxidative enzyme glutathione peroxidase (GSH-Px, iodothyronine desiodinase, selenoprotein P, thioredoxin reductase and the selenoprotein W, that all contain selenocystein. Beside iodothyronine desiodinase that facilitates the activation



of thyroid hormones; all other selenoproteins have a role in the metabolism and detoxification of reactive oxygen species and in the maintenance of the antioxidant defense of cells.

For example, in 1981 the antioxidant treatment of two siblings with DMD, aged six and ten years, was started. At that time the older brother was wheel-chair bound and was not able to walk since eight years of age, the younger one was still able to walk almost normally. The condition of the older brother, who practically had not got carnitine or coenzyme Q<sup>10</sup> supplementation, was gradually deteriorated, and he deceased at 17 years of age. However, at the age of 15 years the younger brother was still able to walk without any assistance. Six months later he became wheel-chair bound. The younger brother was one of the most talented graduated students in his high-school. The mean IQ of DMD patients is without treatment 15-20 points lower than that of normal populations. Anyway for some years he was able to study at the University of Joensuu. Now he is 31 years old and still able to swim some meters, and to produce computer art. Actually he had his first art-show in 2003 in Helsinki. Moreover, only during the nights it is necessary to give him non-invasive breathing assistance.

The present daily megadoses of nutrients supplements (vitamin E 1200mg; sodium selenite 8mg; riboflavin 3mg; pyridoxine 75mg; carnitine 600mg; and coenzyme Q10 180mg) have been well tolerated and no side effects have observed in our open controlled trial (Westermarck et al 1997). The long-term pilot study speaks in favor for a larger study. Dr. Anders Erikson from Sweden (personal communication) has made the same observations with two Swedish siblings suffering from DMD, as we have also made with two 13- and 14-year old cousins 2with DMD.

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